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REVIEW ARTICLE

Prioritization of COPD protein biomarkers, based on a systematic study of the literature

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Abstract: Chronic Obstructive Pulmonary Disease (COPD) is a chronic lung disease mostly due to smoking and until now diagnosed by spirometry (post bronchodilator FEV_1/FVC <70%). However, in spite of the usefulness of FEV_1 as diagnostic and prognostic tool, it has proven to be a weak indicator of future exacerbations, unable to predict lung function decline within COPD patients, as well as unable to identify the smokers "susceptible" to developing COPD at an early stage. Thus, there is an urgent need for biomarkers that address these questions and support clinical decision making in the diagnosis and treatment of (early) COPD. In this respect, considerable efforts have been devoted to identifying protein biomarkers that enable a better understanding of this complex disease and leading to better diagnostic and prognostic tools. However, in spite of the wide range of candidates that have been suggested as potentially useful COPD biomarkers, most remained at the level of the initial discovery, and only fibrinogen has been approved by the Food and Drug Administration (FDA) as predictor for all-cause mortality and COPD exacerbations. There is thus a need for future investigations of these biomarkers in large-scale and well characterized studies in order to prove their usefulness as surrogate endpoints. Based on this, the aim of the present review is to advance COPD biomarker development by providing a comprehensive overview of protein biomarker candidates which have been evaluated in clinical studies and prioritize them according to their potential of becoming valid, clinically useful COPD biomarkers.

Keywords: COPD, biomarker, surrogate marker, review

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Introduction

Chronic obstructive pulmonary disease (COPD) is defined as a 'common preventable and treatable disease that is characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases'[1]. COPD is an umbrella term that covers several pathological conditions and has many clinical and pathological phenotypes[2]. Examples of such phenotypes are chronic bronchitis, emphysema, chronic mucus hypersecretion and small airways thickening. Besides encompassing pulmonary components, this term also covers additional features such as exacerbations and comorbidities, since COPD is recognized as a complex and multicomponent syndrome with both pulmonary and extrapulmonary manifestations[1,3–5]. The forced expiratory volume in 1 second (FEV_1) is the current "gold standard" marker of disease progression, response to therapy, and prediction of mortality[6]. Unfortunately, it has proven to be a weak indicator of future exacerbations, unable to predict lung function decline as well as ineffective for

developing treatment regimens that can significantly reduce mortality rates or alter the disease course[2,7]. For this reason, there has been a growing interest in discovering new biomarkers that would allow a better understanding of this heterogeneous and complex disease[4,8,9]. Interesting challenges in current COPD medicine include developing tools that allow optimizing therapeutic interventions following the shift of a general COPD treatment towards a more personalized, patient-centered medicine[10,11]. For example, biomarkers that would support decisions such as the use of bronchodilators and/or inhaled corticosteroids[12–14], are of outmost interest in order to select the most adequate treatment option for each patient[11]. Furthermore, the prediction of lung function decline amongst COPD patients and therefore development of biomarkers that enable differentiation of slow and fast decliners, would be of importance in developing early stage treatments for particularly the fast decliners as well as in understanding the mechanisms that are involved in the progression and severity of COPD[15–19]. In this respect, it is important to mention that only a small proportion (20–30%) of smokers develop COPD, suggesting that susceptibility to develop the disease is due to the interaction between genetic and environmental factors such as air pollution[20,21]. Being able to recognize individuals at high risk to develop COPD would help clinicians convincing healthy-smokers to quit smoking as well as understanding the genetic basis of the disease.

In COPD, the search for circulating blood biomarkers has mainly focused on plasma/serum proteins which are known to be involved in the systemic inflammatory response to smoking and subsequent lung injury repair[8,15–17,22–26]. In general, cytokines, chemokines, metalloproteases (e.g. MMP-9) and highly abundant acute phase reactants (e.g., C-reactive protein, fibrinogen) have been measured and found to differentiate COPD patients from healthy controls. This directed approach, however, suffers from the fact that proteins not previously suspected to be involved in the disease are not investigated. As a result, several unbiased proteomic studies of plasma and other biological fluids such as induced sputum, exhaled breath condensate, bronchoalveolar lavage or epithelial lining fluid, have been performed in order to find novel protein biomarkers differently expressed in COPD and healthy individuals[9,18,20,23]. While many potential biomarkers candidates have been pointed out, most of them remain at the level of their initial discovery, while only few of them reached the level of clinical validation. In fact, this "biomarker gap" has considerably increased due to the application of proteomic strategies measuring thousands of proteins in groups of samples with 5–20 patients/controls per group. Considering that COPD is a complex and heterogeneous disease, it is unlikely that a single biomarker will be sufficient for COPD diagnosis, prognosis or treatment evaluation. Therefore, Devanarayan *et al.* stated that proteomic strategies should focus on delivering panels of biomarkers which enable the characterization of the different COPD phenotypes as well as reflecting underlying pathological pathways and comorbidities[15]. In line, Agusti *et al.* described a systemic inflammatory network pattern (called inflammome) in subgroups of COPD patients associated with different all-cause mortality and exacerbation frequency[27].

Taking all these into consideration, the aim of the present review is to advance biomarker development in COPD by providing a comprehensive overview of protein biomarker candidates which have been evaluated in large clinical studies, and have the potential of becoming valid and clinically useful biomarkers in the near future.

Methodology and Results

Our search strategy started with the identification of those cohorts with more than 500 subjects and in which biological samples have been collected. For this search, we employed four complementary databases: PubMed, Cochrane Central Register of Controlled Trials, Biomarkers Module from the Thompson Reuters Integrity^{SM} database and ClinicalTrials.gov. The search strategies followed in each case are summarized in Table 1. In all cases, resulting hits were screened, exclusion criteria applied and relevant hits selected. Exclusion criteria were: cohorts with less than 500 subjects; published only as a congress abstract; cohorts without biological samples collected; reviews or literature text-mining publications; studies not focusing on COPD but on dyspnea, bronchiectasis, asthma, or interstitial lung diseases. Based on these criteria we identified 72 cohorts. 52 hits were excluded because genomic or non-protein biomarkers (e.g. desmosine, vitamin D) were investigated, because hits did not have published results yet, or due to double hits in the four different databases. In this way 20 relevant cohorts remained (Table 2). Subsequently, we used PubMed to search for publications reporting protein biomarkers from these cohorts, using the

Table 1. Search strategy for COPD cohorts (>500 subjects, biological samples collected)

Database	Search rules
PubMed	copd[Title] AND cohort [all fields]
Cochrane Central Register of Controlled Trials	#1 copd and biomarker #2 copd and protein #3 copd and serum #4 copd and plasma #5 copd and sputum #6 copd and urine #7 copd and bal #8 copd and elf #9 {or #1–#8}
Thompson Reuters Integrity^{SM} Biomarkers Module	Indication type: COPD Validity: late studies in humans (>500 subjects) Type: proteomic Substrate: DNA exclusion
ClinicalTrials.gov	Search term: COPD Results filtered: - Trials >500 subjects enrolled - observational studies

cohort name or the registration number. From those publications 39 proteins were identified that may have the potential to be relevant biomarkers in COPD (Table 3). Following proposed criteria of Sin *et al*.[2] for developing novel biomarkers in COPD this list was further reduced by selecting only those proteins which showed statistically significant differences between COPD patients and healthy smokers (Table 4). Afterwards this list of 7 prioritized proteins was examined according the other proposed criteria[2] demonstrating: biological plausibility in terms of a role in the pathogenesis of COPD, association with clinical outcomes, evidence that interventions modify the biomarker candidate, and evidence that these changes associate with important clinical outcomes.

COPD Cohorts

Among the identified cohorts (Table 2), a number were observational such as the Bergen COPD study[28,29], PROMISE-COPD[30], the BODE study[31], the MESA Lung study[32] or ECLIPSE[33]. These studies have different aims covering a wide range of aspects related to COPD. PROMISE-COPD aims to investigate whether circulating biomarkers might be able to predict exacerbations during the stable phase of the disease and the clinical outcome of exacerbations in patients with COPD[30]. The BODE study investigated the hypothesis that a multidimensional index (the BODE index) allows a better categorization and prediction of disease outcome (mortality) than FEV_1 alone[31]. The MESA Lung Study is an ancillary study of the Multi-Ethnic Study of Atherosclerosis (MESA) with the aim of testing the chronic endothelial injury hypothesis of COPD and emphysema, in addition to examining subclinical cardiopulmonary interactions[32]. ECLIPSE is a 3-year longitudinal study with four specific aims: (i) definition of clinically relevant COPD subtypes, (ii) identification of parameters that predict disease progression in these subtypes, (iii) examination of biomarkers that correlate with COPD subtypes and may predict disease progression, and (iv) identification of novel genetic factors and/or biomarkers that both correlate with clinically relevant COPD subtypes and predict disease progression[33]. Furthermore, our systematic search identified interventional case-control studies in which different COPD treatments (neutrophil elastase inhibitors, long acting beta agonists, prophylactic antibiotic treatments, cholesterol lowering medication, or corticoidsteroids) were evaluated (NCT00949975[34], NCT01110200[35], NCT00132860[36,37], NCT01061671[38], NCT00325897[39], EUROSCOP[40]). In this category, the Lung Health Study (LHS) is a representative example. The objectives of LHS I and III were (a) to determine whether an intervention program incorporating smoking cessation and the use of inhaled ipratropium bromide could slow down the decline of lung function and reduce the incidence of pulmonary morbidity over a 5-year period of follow-up in persons at high risk for developing COPD, and (b) to estimate the effects on lung function due to bronchodilators over and above the effects of smoking intervention[41]. In LHS II the effect of inhaled corticosteroids (triamcinolone) on the annual age-related decline in pulmonary function in patients with COPD was investigated[42].

Furthermore, we identified and selected cohorts with focus on different lung diseases in which a representative number of COPD patients were recruited. These cohorts are the SAPALDIA study and the European Community Respiratory Health Survey (ECRHS). SAPALDIA was designed to investigate the health effects from long-term exposure to air pollution[43] with the aim of assessing the prevalence of bronchial asthma, chronic (obstructive) bronchitis and allergic conditions in the adult population of Switzerland. An additional aim was the identification of potentially influencing factors, which could be personal (smoking, allergy status, family history, occupation) or environmental (outdoor and indoor pollution, aeroallergens, climate)[44]. The ECRHS aimed to estimate the variation in prevalence of asthma, exposure to risk factors

Table 2. Cohorts identified as relevant for COPD

	Name	Abbreviation	Registration number
1	Bergen COPD cohort study	–	n.a.
2	Predicting Outcome using systemic Markers In Severe Exacerbations of COPD	PROMISE-COPD	ISRCTN99586989
3	Evaluation of COPD to Longitudinally Identify Predictive Surrogate Endpoints	ECLIPSE	NCT00292552
4	The longitudinal study BODE cohort of COPD patients	–	n.a.
5	Endothelial Dysfunction, Biomarkers, and Lung Function -Ancillary to MESA	MESA-Lung	NCT00843271
6	Dose Range Finding Study to Evaluate the Efficacy and Safety of AZD9668 Administered Orally at Three Dose Levels to Patients With COPD on Treatment With Tiotropium	–	NCT00949975
7	COPD Disease Post-hospitalization Study	–	NCT01110200
8	Prophylactic Antibiotic Treatment of Patients With COPD	–	NCT00132860
9	Simvastatin Therapy for Moderate and Severe COPD	STATCOPE	NCT01061671
10	Macrolide Azithromycin to Prevent Rapid Worsening of Symptoms Associated With COPD	MACRO study	NCT00325897
11	European respiratory society study on chronic obstructive pulmonary disease	EUROSCOP	n.a.
12	Lung Health Study (I and III)	LHS I-III, II	NCT00000569/568
13	Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults	SAPALDIA	n.a.
14	The European community respiratory health survey	ECRHS	n.a.
15	Atherosclerosis Risk in Communities Study	ARIC study	n.a.
16	The Malmo preventive project	–	n.a.
17	The Copenhagen cohorts (Copenhagen City Heart Study and the Copenhagen General Population Study)	–	n.a.
18	Cohort of outpatients from West China Hospital of Sichuan University	–	n.a.
19	The Rotterdam study	–	n.a.
20	National Health and Nutrition Examination Survey	NAHNES III	n.a.

for asthma, and asthma treatment across 25 countries from the European Community[45,46]. It is known that COPD can have significant overlap with other lung-related diseases such as asthma[47], making these cohorts useful to investigate the performance of COPD biomarkers in populations with overlapping syndromes. In this line, cardiovascular disease is a well-known COPD co-morbidity which can influence patient mortality and hospitalization rates[48]. Thus, there have been different studies in which protein biomarkers have been investigated in subgroups of COPD patients from cardiovascular disease related cohorts. Examples of this are the ARIC study, a prospective study to investigate the etiology of atherosclerosis and its clinical sequelae and variation in cardiovascular risk factors, medical care, and disease by race, sex, place, and time[49]; the Malmö Prevention Project, a preventive case-finding program for cardiovascular risk factors and alcohol abuse in the city of Malmö[50]; or the Copenhagen City Heart Study[51], a prospective cohort study of individuals aged 20 years and older that were randomly selected from the population of Copenhagen.

Finally, general population cohorts with a relevant (>500) number of COPD subjects were identified and selected including the Copenhagen General Population Study[52], the Rotterdam study (prospective cohort study targeting cardiovascular, endocrine, hepatic, neurological, ophthalmic, psychiatric, dermatological, oncological, and respiratory diseases[53–55]), the National Health and Nutrition Examination Survey (cross-sectional survey providing an estimation of health and nutrition status among a representative group of civilian, noninstitutionalized US residents)[56,57] and the Cohort of outpatients from the West China Hospital of Sichuan University[58].

COPD Protein Biomarkers

After the identification of relevant cohorts, we searched for all publications from these cohorts in which proteins had been investigated or evaluated as potential COPD biomarkers (see Table 3 for the list of proteins). All the proteins on this list have been shown or suggested to be linked to the pathogenesis of the disease, an important *criterium* in the selection of useful biomarkers in COPD[2]. Indeed this list reflects the

complexity of the underlying pathogenesis of COPD, involving inflammation, oxidative stress (e.g., due to smoking), the protease/anti-protease imbalance, environmental factors (such as exposure to pollutants or internal cooking), and genetic predispositions[5,59]. Accumulating evidence indicates that chronic inflammatory and immune responses play key roles in the development and progression of COPD[60,61]. In this respect, most of the potential biomarkers included in our list are proteins involved in inflammation or the immune response such as CRP, IL-6, beta defensin 2, among many others[25–27,29,36,37,54,62–74]. One of the main causes leading to COPD is chronic smoking which exposes the respiratory tract to reactive oxygen species, resulting in oxidative stress and injury (which in the last instance leads also to inflammation)[59,75]. Thus, biomarkers of oxidative stress such as ceruloplasmin[68] could be used to reflect this characteristic of COPD. Furthermore, one of the main components of COPD is emphysema which is caused by an imbalance between proteases and anti-proteases resulting in lung parenchymal destruction. Representative molecules of this process are MMP's[66,72] involved in the breakdown of the extracellular matrix and in tissue remodeling, as well as, VEGF involved in angiogenesis and tissue remodeling[72]. Furthermore, our protein list comprises lung-specific proteins such as SPD and CC-16[76–79]. CC-16 is secreted by non-ciliated club cells (formerly known as Clara cells) which are mainly present in the respiratory bronchioles and by non-ciliated columnar cells present in the small airways. SPD is found in the endoplasmic reticulum of type II pneumocytes and in the secretory granules of club cells. SPD plays a role in the protection against viral infections, the clearance of bacteria, fungi and apoptotic cells, as well as in inflammation[80]. Finally, proteins that enable to investigate the effect of long-term inhaled corticosteroid therapy on bone mineral density (a clinical feature in COPD) such as osteocalcin were identified[81,82].

Table 3. Proteins investigated as potential COPD biomarkers within relevant COPD cohorts

Protein name	Abbreviation	ID	Proposed pathophysiological role	Reference
Adiponectin		Q15848	Anti-inflammatory, anti-diabetic, and anti-atherogenic activities	[64,66,105]
Beta-defensin-2		A4H1Z7	Role in pulmonary immunity	[66]
Calprotectin		P05109	Anti-infective and anti-inflammatory activities	[36]
Chitinase3-like-1	YKL-40	P36222	Role in cell proliferation and differentiation , inflammation, extracellular tissue remodeling, and protection against apoptosis	[37]
Club cell protein 16	CC-16	P11684	Pulmonary inflammatory protein	[22,26,35,78,79,86,91,106,107]
C-C motif chemokine 18	PARC/CCL-18	P55774	Lung-predominant inflammatory protein.	[66,70,72,73,86–88,91,107,108]
C-C motif chemokine 2	CCL2	P13500	Inflammatory chemokine	[66]
C-X-C motif chemokine 16	CXCL 16	Q9H2A7	Inflammatory chemokine	[29]
Ceruloplasmin		P00450	Protein involved in oxidative stress	[68]
C-reactive protein	CRP	P02741	Secreted by the liver in response to a variety of inflammatory cytokines	[22,26,27,29,34,38,52,58,64,66,69,70,84,86–91,93–95,106–115]
C-X-C motif chemokine 10	CXCL-10	P02778	Inflammatory chemokine	[66]
E-selectin		P16581	Cell adhesion molecule expressed only on endothelial cells activated by cytokines.	[62]
Fibrinogen		Q08830, P02671, P02675	Coagulation factor	[22,26,27,64,66,68,70,83–91,106–108,111,114,116]
Fibronectin to CRP ratio		-	Coagulation factor/ secreted by the liver in response to a variety of inflammatory cytokines	[69]
Haptoglobin		P00738	Inflammatory role	[68]
Hepatocyte growth factor		P14210	Systemic inflammation marker	[66]
Interleukin-1 beta	IL 1β	P01584	Proinflammatory cytokine	[67]
Interleukin-6	IL 6	P05231	Inflammatory interleukin	[22,26 27,34,58,64,66,67,70,72,84,86,91,95,107,111,114,115]
Interleukin-8	IL 8	P10145	Inflammatory interleukin	[22,26,27,34,66,70,72,84,86,91,107,115]

Continued 3

Protein name	Abbreviation	ID	Proposed pathophysiological role	Reference
Interleukin-12 subunit beta	IL-12β	P29460	Inflammatory interleukin	[66]
Pro-interleukin-16	IL 16	Q14005	Inflammatory interleukin	[72]
Leptin		P41159	Role in lung maturation and development	[64]
Leukotriene A-4 hydrolase		P09960	Inflammatory marker	[74]
Mannose binding lectin	MBL	P11226	Collectin involved in host defense against infection	[63,117]
Neutrophil collagenase	MMP-8	P22894	Breakdown of extracellular matrix involved in tissue remodeling	[66]
Matrix metalloproteinase-9	MMP-9	P14780	Breakdown of extracellular matrix involved in tissue remodeling	[66,72]
Monocyte chemoattractant protein	MCP-1/CCL2	P13500	Chemokine regulating migration and infiltration of monocytes/macrophages	[72,112]
Myeloperoxidase	MPO	P05164	Cytotoxic protein used by neutrophils to kill bacteria and other pathogens	[66,71]
Neutrophil gelatinase associated lipocalin	NGAL	P80188	Inhibition of bacterial growth and enhancement of matrix degradation	[113,118]
Orosomucoid	AGP1	P02763	Inflammatory marker	[68]
Osteocalcin		P02818	Indicator of bone mineral density	[81,82]
Osteoprotegrin/Tumor necrosis factor receptor superfamily member 11B	OPG	O00300	Is a decoy receptor for the receptor activator of nuclear factor kappa B ligand (RANKL)	[52,54,119]
soluble intercellular adhesion molecule	ICAM-1	P05362	Facilitates leukocyte adhesion and migration across the endothelium	[62]
Soluble receptor for advanced glycation endproducts	sRAGE	Q15109	The RAGE pathway has been shown to be associated with several inflammatory diseases	[65,91]
Soluble tumor necrosis factor receptor 1	sTNF-R1	P19438	Proinflammatory cytokine	[52,54,119]
Surfactant protein D	SPD	P35247	Pulmonary protein involved in innate immunity	[22,26,35,70,76,77,80,86,87,91,106–108,115]
Tumor necrosis factor	TNFα	P01375	Proinflammatory cytokine	[22,26,27,34,58,67,70,72,84,86,91,107]
Vascular endothelial growth factor	VEGF	P15692	Angiogenic protein/marker of injury and repair	[72]
Vitamin D binding protein	DBP	Q6LDC6	It binds to vitamin D and its plasma metabolites and transports them to target tissues	[106]

Prioritized COPD Protein Biomarkers

The list of potential protein biomarkers (Table 3) was further reduced by selecting only those that show statistically significant differences between COPD patients and healthy smokers (Table 4). This follows the recommendation to demonstrate a strong, consistent and independent association between a biomarker and COPD[2]. In this way, 7 potential proteins remained: fibrinogen, CRP, IL-6, PARC, SPD, CC-16, and sRAGE.

Among these proteins, fibrinogen was recently approved as biomarker for all-cause mortality and exacerbations in COPD subjects. Fibrinogen reflects the systemic inflammatory component of COPD, is elevated in COPD patients[27,64,66,83–85], and associated with the severity of the disease[27] and FEV_1[26]. Furthermore, fibrinogen is associated with increased exacerbation frequency, hospitalization, and mortality in COPD patients[66,68,83,86–88], as well as with increased cardiovascular events[70,89–91]. Therefore, fibrinogen has been accepted by the FDA and COPD Biomarker Qualification Consortium (CBQC) for the stratification of subjects at risk for hospitalization and mortality[92]. CRP and IL-6 have also been discussed within the CBQC as interesting COPD biomarkers since they share many of the features of fibrinogen. CRP and IL-6 also reflect the systemic inflammatory component of COPD, are increased in COPD patients and are associated with important clinical outcomes[27,29,52,64,66,70,84,86–90,93–95]. However, the biological variability of fibrinogen was lower than for IL-6 or CRP, which both display a wide variability in stable COPD subjects[66,92].

The proteins SPD, CC-16 and PARC are promising COPD biomarkers, particularly because they are

Table 4. Prioritized proteins able to discriminate between COPD patients and healthy smokers

Protein name	Abbreviation	Protein ID	Sample	Concentration in smoker controls	Concentration in COPD patients	Significance (p-value)	Reference
Club cell protein 16	CC-16	P11684	serum	5.6 (3.1) ng/ml	4.9 (3.4) ng/ml	<0.001	[78]
C-C motif chemokine 18	PARC/CCL-18	P55774	serum	81 (21) ng/ml	105 (26) ng/ml	<0.0001	[73]
C-reactive protein	CRP	P02741	serum	1.6 (0.8–3.3) µg/ml	3.2 (1.5–7.1) µg/ml	<0.001	[27]
Fibrinogen		Q08830, P02671, P02675	EDTA plasma	391 (348–436) mg/dl	448 (388–517) mg/dl	<0.001	[27]
Interleukin-6	IL 6	P05231	serum	0.6 (0.3–1.3) pg/ml	1.5 (0.8–3.1) pg/ml	<0.001	[27]
Soluble receptor for advanced lycation endproducts	sRAGE	Q15109	serum	1.7 (0.7) ng/ml	1.4 (0.6) ng/ml	<0.001	[65]
Surfactant protein D	SPD	P35247	Serum	114(76–162) ng/ml	121 (85–174) ng/ml	0.021	[80]

Concentrations are presened as mean (standard deviation) or median (interquartile range).

highly lung specific. PARC was shown to be elevated in COPD patients when compared to healthy smoking controls[66,73], and an association between the levels of PARC and body mass index, FEV_1, BODE index, and exacerbation rate was observed[24]. However, in later work only a statistically relevant association with the BODE index was reconfirmed[72]. Furthermore, elevated blood PARC levels have been associated with a higher risk of mortality and exacerbation episodes in COPD patients[86–88]. The largest study of PARC in COPD to date is the one performed by Sin and co-workers[73]. They observed that a 2-week treatment with prednisolone associated with reduced PARC levels when compared to placebo. Furthermore, a relationship of PARC concentrations with a future risk of cardiovascular hospitalization and mortality and with total mortality was observed. However, some of the results of this study were contradictory: in the LHS study higher PARC levels were associated with lower baseline FEV_1 values and increased mortality due to cardiovascular disease, while in the ECLIPSE subjects the association with FEV_1 could not be replicated. Finally, PARC levels show wide variability over time both in stable COPD patients and healthy controls[66]. Thus, additional work is needed to determine the utility of this protein as COPD biomarker.

Amongst the lung specific proteins, SPD and CC-16 have proven to be more stable over time than PARC which could make them more useful COPD biomarkers[66]. SPD is mainly produced in type II pneumocytes[96,97] and plays an important role in pulmonary immune defense[98], making it an interesting potential biomarker in COPD. In this respect, polymorphisms in the surfactant protein–D gene (SFTPD) have been shown to be associated with susceptibility to develop COPD and to influence serum concentrations of SPD[22,77]. Furthermore, SPD levels were found to be elevated in COPD patients when compared to healthy smoking controls, although no association with COPD severity[80] nor with the rate of change in FEV_1[26] was observed. SPD has also proven to be associated with a higher risk for exacerbations[80,87] and mortality[86] as well as being reduced after prednisolone treatment[80]. SPD has different isoforms including a nitrosylated and a cleaved form[76], however, these modified SPD products associate worse with clinical outcomes of COPD than non-modified SPD. Although considered a promising COPD biomarker, SPD has so far not proven to be a reliable prognostic tool requiring further validation of the findings observed so far. CC-16 is predominantly secreted from non-ciliated club cells and is localized in terminal and respiratory bronchial epithelia[99]. Significant associations between several single nucleotide polymorphisms and circulating levels of CC-16 have been identified in genome-wide association studies, however, no strong associations between genotypic variations, circulating CC16 levels and risk for developing COPD were observed[22]. CC-16 is decreased in COPD patients when compared to healthy smoking controls[78] and has been significantly and independently associated with the rate of change in FEV_1[26]. However, CC-16 levels were not significantly associated with mortality[86]. Furthermore, changes in CC-16 levels are not specific to one agent, disease state or specific exposure[100]. As CC-16 is likely to be affected by other forms of lung disease the biomarker role of CC-16 in COPD is uncertain and additional data is required to assess more rigorously its potential as surrogate for disease phenotype, severity and/or progression.

sRAGE, the soluble form of the receptor of ad-

vanced glycation end-products (RAGE), and a multi-ligand member of the immunoglobulin superfamily of cell surface molecules, has been associated with several diseases including diabetes mellitus, cardiovascular, and respiratory diseases[101–103]. RAGE is expressed in a variety of tissues including the lung. However, unlike in other tissues, RAGE is highly expressed in the lung under normal physiological conditions[101]. Increased expression of RAGE was demonstrated in the proximal and distal airways of COPD patients compared to both smoking and non-smoking controls, and these levels correlated positively with impaired lung function[104]. sRAGE is the resulting product of both alternative splicing (esRAGE) and cleavage of membrane-bound RAGE[101,102]. Circulating levels of sRAGE are decreased in COPD patients when compared to healthy smoking controls and appear to be associated with emphysema independently of COPD severity[65]. In addition to the association of sRAGE with emphysema, two SNPs in the gene coding for RAGE (AGER locus) were significantly associated with systemic sRAGE levels. These results indicate that sRAGE is a very promising COPD biomarker that deserves further investigation in order to prove its usefulness in COPD diagnosis, prognosis and treatment development.

It is worth mentioning that all of the described studies used ligand binding assays (LBAs; mainly ELISA), often from the same supplier, to quantify proteins in biological matrices. This bears the risk that forms of a given protein that are recognized by the antibody(ies) generate signals while others do not, meaning that our picture of how these biomarker candidates reflect disease development is likely incomplete. There is further a risk that systematic bias due to cross-reactivity of the antibodies with other proteins may affect the results. LBAs are generally not suitable to discriminate between different forms of a protein due to genetic variability (e.g., due to splicing, single amino acid variation, different allele distribution) or post-translational modifications (e.g. cleavage by proteases, modifications due to oxidative stress). Alternative methodologies based on chemical analysis principles such as LC-MS may provide a different, more detailed picture of protein heterogeneity with possibly better sensitivity and specificity and predictive values for the various aspects of COPD.

Conclusion

While many protein biomarker candidates have been suggested for COPD diagnosis and prognosis, so far only fibrinogen has been approved by the FDA for some aspects of the disease. Validation of promising biomarkers (for example those in Table 4) in well-designed studies, taking into account the different phenotypes of COPD, is thus urgently needed to reduce the gap between initial discovery and clinical application. Most of the studies performed so far comprise observational cohort and small-sized experimental studies, while large randomized clinical trials (e.g. evaluating changes in biomarkers in relation to interventions and clinical outcome) are scarce. Most of the current biomarker candidates are not lung-specific but may reflect the systemic aspects of COPD and its associated co-morbidities. There is a need for lung-specific biomarkers derived from proteins that are either only expressed in the lung (CC-16, SPD), or highly over-represented in the lung (RAGE). Because of the complexity of COPD, it is not likely that a single biomarker will be able to address the different aspects (phenotypes) of the disease. In this respect, a panel of biomarkers may be needed to guide personalized treatments of COPD patients[27].

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